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Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers

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Abstract

Purpose To evaluate the prognostic value of androgen receptor (AR) expression in patients with estrogen receptor (ER)-positive breast cancer treated with endocrine therapy with or without the addition of chemotherapy.

Patients and Methods A consecutive series of 953 patients with ER-positive breast cancer treated between 1998 and 2003 was selected. Repeated immunohistochemistry confirmed the expression of ER in the tumor of 938 patients. AR expression was measured by immunohistochemistry. The Kaplan-Meier method, logrank test and multivariate Cox models were used to explore the impact of AR expression on time to relapse (TTR) and disease specific survival (DSS) in all patients and in subgroups treated with chemo-endocrine therapy or endocrine therapy alone. *Results* AR immunoreactivity was assessable in 859 tumors and positive in 609 (70.9%). AR expression was a significant marker of good prognosis for TTR ($P=0.001$) and DSS ($P<0.001$). This effect was particularly evident in the group of patients receiving chemo-endocrine therapy (TTR ($P=0.015$) and DSS ($P<0.001$)). Cox models confirmed AR as an independent variable for both TTR ($P=0.003$, HR 0.444 , 95%CI 0.258-0.765) and DSS ($P<0.001$, HR 0.135, 95%CI 0.054-0.337). Thus, we focused on ER positive luminal B breast cancer that may be selected for chemotherapy because of their more aggressive immunophenotype. In this subset AR expression identified a group of patients with better prognosis for TTR ($P=0.017$, HR 0.521, 95%CI 0.306- 0.888) and DSS ($P=0.001$, HR 0.276, 95% CI 0.130- 0.588). *Conclusion* AR expression is an independent prognostic factor of better outcome in patients with ER-positive breast cancers

Keywords: Androgen receptor, prognosis, estrogen receptor, breast cancer, chemotherapy.

Introduction

Increasing data support a possible role of the androgen receptor (AR) as a marker of prognosis [1,2], particularly among patients with estrogen receptor (ER) positive breast cancer [3]. AR are frequently expressed together with ER and progesterone receptor (PgR) in well differentiated breast cancer [4-6], but are rarely present in human epidermal growth factor receptor 2 (HER2) positive tumors [7,8]. Specific cancer histotypes, such as the apocrine carcinoma consistently express AR [9-10] despite being ER and PgR-negative, and AR expression has been reported in about 45% of triple negative breast cancers [4,8]. Studies using different analytical procedures such as a binding assay [11], immunohistochemistry (IHC) [3,12] and reverse-phase protein arrays [13] have suggested a possible role of AR to predict responsiveness to endocrine therapy. The impact of AR expression on survival of patients with high risk ER-positive breast cancer, such as luminal B breast cancer [14-16], has never been evaluated. However, it has been shown that in metastatic breast cancers, the median survival after disease recurrence of patients with AR-expressing tumors was significantly longer compared to that of patients with AR-negative tumors [2].

In this study, we evaluated the prognostic value of AR in a large series of patients with ER positive breast cancer after long follow-up. All patients were treated with endocrine therapy, with or without addition of chemotherapy. In particular, we focused on a subset of patients with tumors closely similar to luminal type B by conventional markers [17] and considered as more clinically aggressive than the luminal type A ER-positive breast cancers.

Patients and methods

Study design

A consecutive series of 953 patients diagnosed with ER-positive breast cancer between 1998 and 2003 was retrieved from the files of the Pathology Departments of the San Giovanni Battista-

Molinette Hospital (431 cases) and S. Anna Hospital (522 cases) in Turin, Italy. Tumor slides were centrally reviewed by two of the authors (AS, IC). Representative blocks were obtained and multicore tissue microarrays (TMAs) were prepared, as previously described [18], using the advanced tissue arrayer (mod ATA-100, Chemicon International, Temecula, CA, USA). Sections of the resulting recipient blocks were retested by IHC for ER status. Fifteen of 953 tumors were deemed ER-negative on TMAs and the negative results were further confirmed on whole tissue sections. These cases were excluded from the study (Fig. 1). Clinical data of the remaining 938 patients including age, type of surgery, type of treatment, occurrence and type of relapse and current status were obtained from the Oncology Department of the two institutions. To confirm accuracy of previously recorded data, medical charts of all patients were reviewed. Pathological data including histotype, grade, and size of the tumors, peritumoral vascular invasion and stage were recorded according to the original diagnosis. The study was conducted in compliance with the ethical regulatory issues of the participating institutions.

Immunohistochemical procedure

IHC was performed using an automated slide processing platform (Ventana BenchMark AutoStainer, Ventana Medical Systems, Tucson, AZ, USA) and the following primary antibodies: prediluted anti-ER rabbit monoclonal antibody (SP1, Ventana-Diaphat, Tucson, AZ, USA); prediluted anti-PgR rabbit monoclonal antibody (1E2, Ventana-Diaphat); anti-AR mouse monoclonal antibody (AR441, diluted 1:50, Dako, Glostrup, Denmark); anti-Ki67 monoclonal antibody (MIB1, diluted 1:100 Dako) and anti-HER2 polyclonal antibody (A0485, diluted 1:800, Dako). FISH assays for assessing HER2 gene amplification were performed for IHC equivocal (score 2+) cases, as previously reported [19].

Positive and negative controls (omission of the primary antibody and IgG-matched serum) were included for each immunohistochemical run.

All TMA slides were scanned by the Aperio system (ScanScope CS System, Vista, Ca, USA) and automated counting was performed. To ensure the reliability of the automatic assessment all cases were reviewed on screen by two of the authors (A.S. and I.C.) blinded to patients' treatment assignment or outcome. If the recorded percentage of immunostained cells differed by more than 10%, then a collegial re-evaluation of the results was performed by three pathologists at the multiheaded microscope (I.C., R.A. and A.S.). This occurred for 1.2% of tumors. According to previous studies the cut off value for ER and PgR positivity was set at $\geq 1\%$ and the same cut-off was also adopted for AR positivity [20]. The percentage of Ki67-positive cells was recorded and the cut-off for dichotomizing tumors with low and high proliferative fraction was established at 10% positive cells for the overall study population, and at 14% for sorting out luminal type B breast cancer as suggested by Cheang MC et al [17]. HER2 status was classified as negative or positive (when scored 3+ by IHC or *HER2* amplified by FISH) according to the recommended guidelines for invasive carcinoma [21].

Statistical Analysis

Differences between AR positive versus AR negative tumors were analyzed by univariate analysis with the Chi-square or Fisher's exact test: *P*-values < 0.05 were considered to be statistically significant.

Median follow-up time was calculated as the median observation time among all patients. Follow-up was censored at the time of death or the last clinical investigation of the patient.

Disease-specific survival (DSS) was calculated from the data of definitive surgery to the date of death of disease. Patients dying from other causes were censored at the time of death. Time to relapse (TTR) was measured from the data of definitive surgery to the data of first recurrence (defined as loco-regional or systemic). Contralateral breast cancer was not considered as relapse.

The Kaplan-Meier method was used to estimate the TTR and DSS. The logrank test was used to test the survival differences.

Univariate analysis was used to examine what variables were associated with prognosis. Variables of interest included: age, histotype, histological grade, pT and pN staging, vascular invasion, type of surgery (mastectomy *versus* breast conserving surgery), radiotherapy and chemotherapy. In order to adjust the risk estimate for potential confounders, a Cox proportional hazard regression for DSS and TTR was used and hazard ratios (HR) were calculated. Multivariate analysis included variables significant at $P < 0.05$ in univariate models or with a priori hypothesis for inclusion.

Statistical analysis was carried out using the SPSS version 17 (SPSS Inc, Chicago, IL) software, the R environment [www.r-project.org], SAS version 9.1 (SAS Institute, Cary, NC) and S-PLUS version 6.1 (Insightful Corp, Seattle, WA). This article was written in accordance with Reporting Recommendations for Tumor Marker Prognostic Studies guidelines [22].

Results

Patients' characteristics

The study cohort included 938 patients with tumors confirmed as ER positive. Clinical and histopathological features of the whole population are reported in Table 1. The median follow-up time was 6.8 years.

All patients were treated with endocrine therapy. The majority received tamoxifen as first option, 180 patients switched from tamoxifen to an aromatase inhibitor after 2 years. Using the local protocol (Online Resource 1) defined during the time period of this study (patient's age <50 years and tumor >2 cm in size and/or presence of lymph node metastases and/or presence of peritumoral vascular invasion), a cohort of 390 patients received both endocrine and chemotherapy.

Androgen receptor expression and outcome

AR was assessable in 859 tumors and 609 (70.9%) of these were considered positive. As shown in Table 2, AR positivity was associated with small tumor size (<2 cm), absence of lymph node metastases and PgR expression. Of the 250 AR-negative cases, 137 (54.8%) were treated with endocrine therapy only and 113 (45.2%) with endocrine and chemotherapy; of the 609 AR positive cases 358 (58.7%) were treated with endocrine therapy only and 251 (41.2%) with endocrine and chemotherapy.

Univariate analysis performed on the whole cohort of patients confirmed the correlation of the traditional histological and immunophenotypical factors with TTR (Table 3) and DSS (Table 4). Focusing on AR expression, 609 patients with AR-positive tumors had a better outcome (Fig. 2a and 2b) (TTR: $P=0.001$ and DSS: $P<0.001$). Then we evaluated the effect of AR expression in the sub-cohort of patients treated with endocrine therapy alone. In this subset the P -value of AR positivity was marginally significant at 0.046 for TTR (Table 3) and not significant for DSS ($P=0.247$; Table 4; Fig. 3a). By contrast, in the group of patients receiving endocrine and chemotherapy, AR positivity was a strong prognostic factor for both TTR ($P=0.015$; Table 3) and DSS ($P<0.001$; Table 4, Fig. 3b). Following this result, we evaluated the effect of AR according to the different chemotherapeutic regimens, and we observed that AR expression was a marker of good prognosis in the group of patients treated with cyclophosphamide, methotrexate, and fluorouracil (CMF) ($P=0.001$) and anthracycline ($P= 0.018$) (Fig. 3c and 3d). While no such significance was demonstrated among subsets of patients receiving other types of chemotherapy the numbers were limited and the data do not establish a reliable difference.

Cox multivariate analysis applied on the whole series of ER-positive cases for both TTR and DSS confirmed that AR status was an independent prognostic factor (Table 5) . The same role of AR was observed at Cox analysis for TTR, but not for DSS in patients treated with endocrine

therapy alone (Table 6), while AR remained an independent factor of good prognosis for both TTR and DSS in the group of patients treated with endocrine and chemotherapy, as did PgR and HER2 (Table 7). However, a formal test for interaction between the use of chemotherapy and the expression of AR was not significant either for TTR ($P=0.66$) or DSS ($P=0.21$) (Fig. 4), so this apparent difference needs to be interpreted with caution.

Androgen receptor expression and outcome in luminal type B breast cancer

To further explore the prognostic value of AR in ER-positive breast cancer with more aggressive biology that may be the target of chemotherapy, we selected a series of 343 cases classified on the basis of immunohistochemical markers as closely similar to the genetically defined Luminal B, as recently proposed by Cheang M.C. et al [17]. Briefly, we considered as Luminal B HER2 positive tumors and HER2 negative tumors with Ki67 >14%. Comparison of DSS and TTR of luminal type B against luminal A tumors confirmed poorer outcome among patients classified as Luminal B (Fig. 3e). Univariate analysis showed that the prognostic value on DSS of AR was maintained within the luminal B tumors (Fig. 3f) and Cox analysis confirmed this result for AR together with age, tumor size, vascular invasion and chemotherapy (Table 8).

Discussion

In the present study on a large series of ER-positive breast cancers, AR expression, as evaluated by IHC, provides statistically significant prognostic value beyond established clinico-pathological parameters.

The role of AR as a prognostic marker has been suggested by other authors, though there is a high variability in patient populations, assay methods, analysis of results, definitions used and reporting of results [3,10-12,23]. Particularly, the IHC cut-off values for positive and negative AR status are quite variable within different studies [3,8] or are not reported at all [12]. In the present

study we used an automated system of analysis of IHC reactions that should avoid the bias of subjectivity linked to quantitative evaluation of IHC markers as performed by traditional microscopy. Using a cut off of $\geq 1\%$, the AR expression rate in ER-positive breast cancer was of almost 71%, which is comparable with previous reports on breast cancer in general [4,8]. If the cut-off was increased to $\geq 10\%$, the prevalence of positive cases would decrease to 60%, though the significant correlation with outcome is maintained (Online Resource 2). A possible bias of the present work might be the use of TMAs, but using the multicore procedure we have previously shown that hormone receptors -and in particular ER levels- are correctly evaluated without significant discrepancy as compared to whole section analysis [18].

In addition, we showed that positive AR expression by IHC identifies patients with better prognosis within the subset of luminal type B tumors. Gene expression studies have identified two molecularly distinct subtypes of ER-positive breast cancers, the luminal types A and B, with luminal B tumors having poorer outcomes than luminal A tumors [14-16]. Recent studies have suggested that a combination of conventional IHC markers [17] may be considered a suitable surrogate for identifying a subset of breast cancers closely similar to the luminal B type defined by gene expression analysis. Our results show that in luminal type B cancers AR expression could be used as a prognostic marker, selecting a group of AR-positive tumors with better prognosis.

The likelihood of response of ER-positive breast cancers to adjuvant systemic chemotherapy cannot be accurately predicted, and the results of our retrospective study do not allow any conclusion that AR expression can predict which patients will benefit from the addition of chemotherapy, or indeed any particular type of chemotherapy, though the information that overall risk of relapse is lower in patients whose tumors express AR may assist decision-making in some cases. However, a recent study of triple negative breast cancers has shown by in vitro ATP-based chemotherapy response assay (ATP-CRA) that chemosensitivity to 5-fluorouracil and methotrexate was higher for tumors expressing AR and that this effect was not observed with other chemotherapeutics, such docetaxel, doxorubicin, epirubicin, vinorelbine, gemcitabine, oxaliplatin

and paclitaxel [24]. Although a statistically significant interaction between chemotherapy and the expression of AR was not demonstrated, our results confirmed both by uni- and multivariate analysis a significant correlation of AR positive immunoreaction and longer DSS in patients treated with any chemotherapeutic regimen containing CMF and also with regimens containing an anthracycline, while there was not a significant correlation of AR with survival in patients treated with taxanes or vinorelbine. On the other hand, recent experimental data suggest a role for AR overexpression as a novel mechanism of tamoxifen resistance and speculate that AR and ER could collaborate to regulate cyclin D1 gene expression, thereby promoting cell cycle progression in the presence of tamoxifen [25]. Taken together these data may suggest that AR/ER positive breast cancer with high Ki67 expression could resist to tamoxifen therapy and benefit from specific chemotherapeutic treatment containing CMF and/or anthracyclines. Specific clinical trials and independent data set of patients with ER positive disease who received chemo-endocrine therapy are needed to confirm this hypothesis.

The 2009 St. Gallen recommendations consider endocrine therapy indicated if any ER staining is present in the tumor [26]. One of the most difficult challenges in treating these patients, however, is the identification of patients with incompletely endocrine-responsive tumor who should receive a chemo-endocrine treatment. In prostate cancer, it has been suggested that knowledge of AR action in promoting cell proliferation can be used to design strategies that maximize cell death in response to cytotoxic therapy [27]. The results of the present study suggest that AR could be an important parameter for designing specific systemic treatments for the patients with ER-positive breast cancer.

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Figure Legends

Fig. 1 Study flowchart shows the process for patient selection

ER Estrogen Receptor; *TMA* tissue micro array.

Fig. 2 Kaplan-Meier estimates of (a) time to relapse and (b) disease specific survival according to androgen receptor in 859 ER-positive breast tumors

Fig. 3 Kaplan-Meier estimates of disease specific survival according to androgen receptor in patients treated by (a) endocrine therapy, (b) endocrine and chemotherapy, (c) Cyclophosphamide, Methotrexate and Fluorouracil (CMF) and (d) Anthracyclines. Kaplan-Meier estimates of disease specific survival according to (e) luminal types of breast cancers and according to (f) androgen receptor in luminal B cases

AR. Androgen Receptor; *DSS*. disease specific survival; *CI*. confidence interval

Fig. 4 Proportional hazard model results of Cox analysis in the whole ER-positive cohort and in the endocrine treated and endocrine and chemo-treated subgroups

HR. hazard ratio; *CI* confidence interval.

Supplementary material

Online resource 1: Age and pathological data according to chemotherapy (CT) treatment

Article title: Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers

Journal name: Breast Cancer Research and Treatment

Author names: Isabella Castellano, Elena Allia, Valeria Accortanzo, Anna Maria Vandone, Luigi Chiusa, Riccardo Arisio, Antonio Durando, Michela Donadio, Gianni Bussolati, Alan S. Coates, Giuseppe Viale, Anna Sapino

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Online resource 2: Kaplan-Meier estimates of time to relapse (a) and disease specific survival (b) in 859 patients according to androgen receptor cut off of 10%

AR: Androgen Receptor; DSS: disease specific survival; TTR: time to relapse; CI: confidence interval

Article title: Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers

Journal name: Breast Cancer Research and Treatment

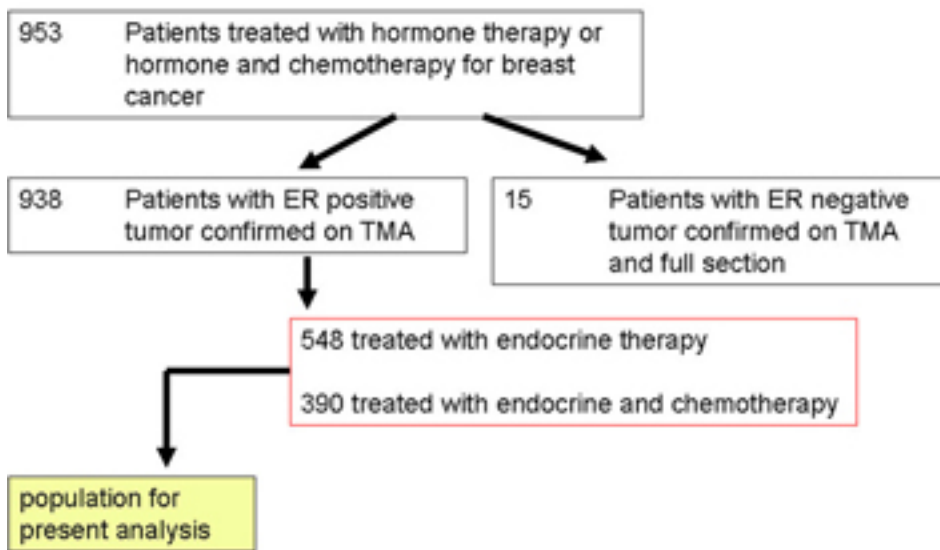
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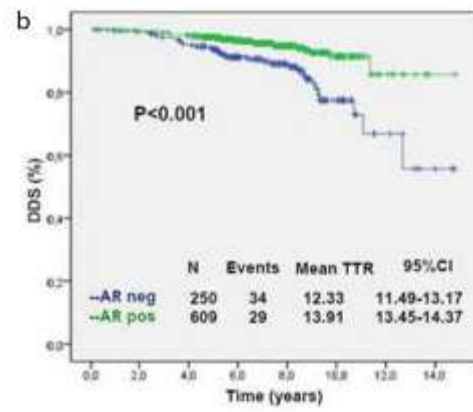
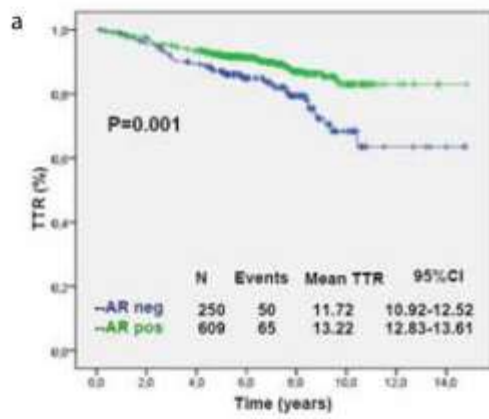
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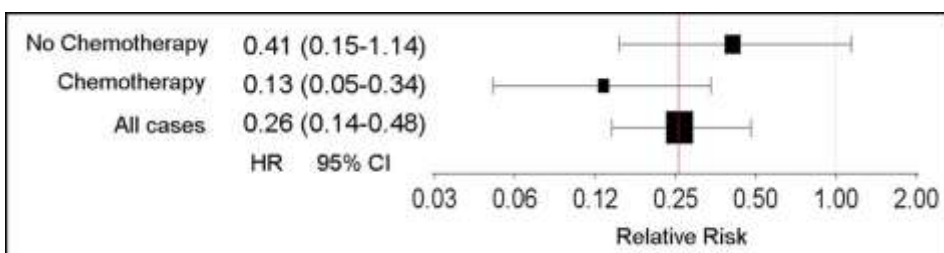
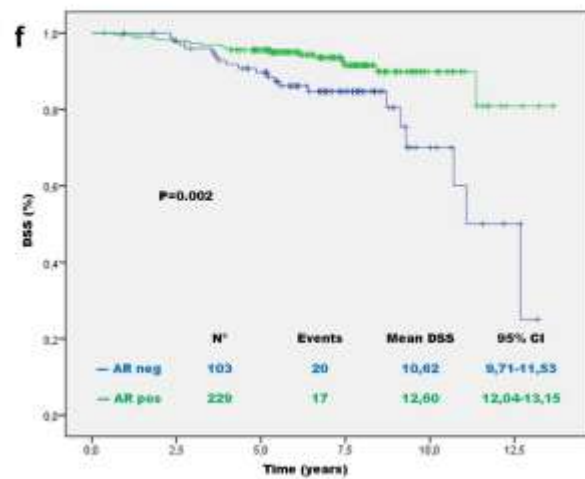
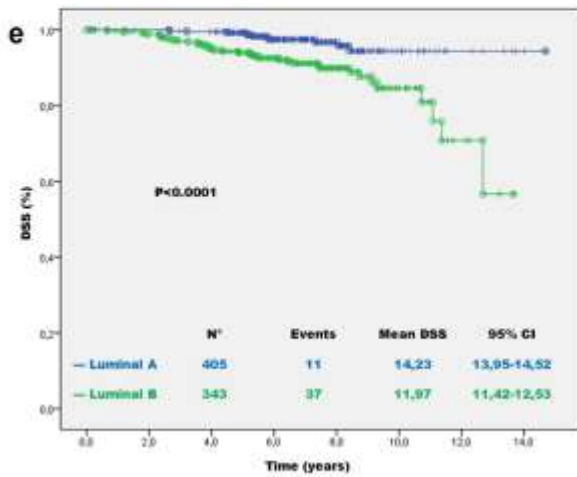
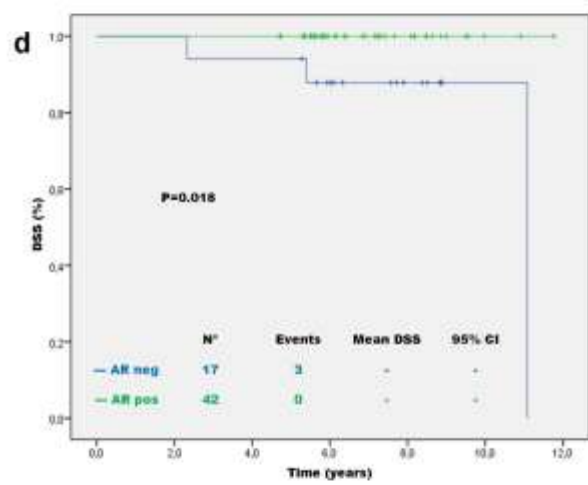
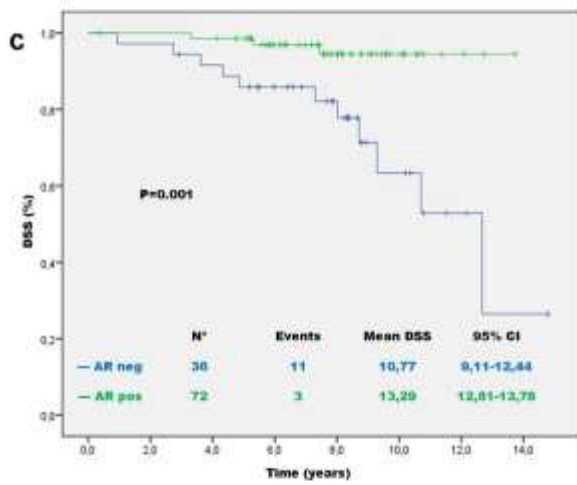
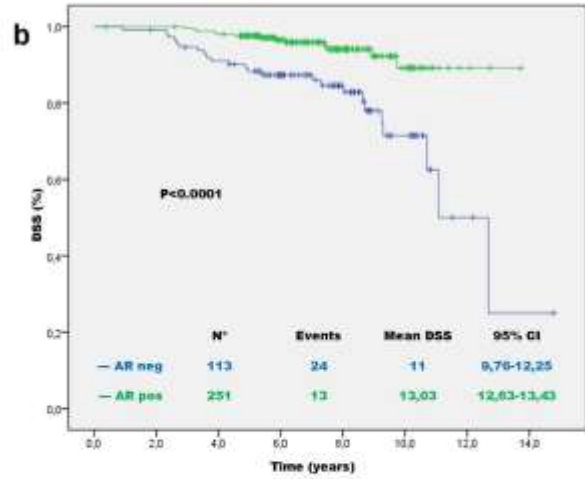
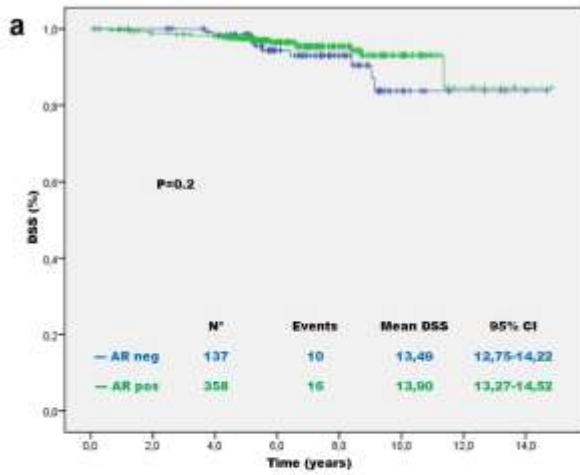


Table 1 Clinical and histopathological characteristics of 938 estrogen receptor positive breast cancers

	Number of cases	Total %	Valide %
Age			
≤ 40 years	31	3.3	3.3
40-70 years	717	76.4	76.4
≥ 70 years	190	20.3	20.3
Relapse Type			
No	791	84.3	84.3
Local	35	3.7	3.7
Systemic	90	9.6	9.6
Controlateral	22	2.3	2.3
Death of disease			
No	868	92.5	92.5
Yes	70	7.5	7.5
Histotype			
Ductal and Lobular	638	68.0	68.0
Special types	300	31.9	31.9
Grade			
1	315	33.6	33.6
2	406	43.3	43.3
3	217	23.1	23.1
pT			
1	600	64.0	64.0
2	282	30.1	30.1
3	26	2.8	2.8
4	29	3.1	3.1
x	1	0.1	0.1
pN			
0, ITC, IMIC	508	54.2	62.0
1,2,3	311	33.2	38.0
Unknown	119	12.7	
Type of surgery			
Mastectomy	255	27.2	27.2
Conservative	683	72.8	72.8
Vascular invasion			
No	429	45.7	45.7
Yes	509	54.2	54.2
Radiotherapy			
No	258	27.5	27.5
Yes	680	72.5	72.5
Chemotherapy			
No	548	58.4	58.4
Yes	390	41.6	41.6
Type of endocrine therapy			
Tamoxifen	709	75.5	75.5
Switch	180	19.1	19.1
Aromatase Inhibitors	49	5.2	5.2
Type of chemotherapy			
CMF	112	28.7	28.7
Anthracycline	66	16.9	16.9
FEC or CMF+FEC	116	29.7	29.7
Taxanes or Vinorelbine	96	24.6	24.6

ITC isolated tumor cells; *MIC* micrometastasis; *CMF* cyclophosphamide, methotrexate and fluorouracil; *FEC* fluorouracil, epirubicin and cyclophosphamide

Table 2 Patients' and histopathological characteristics according to androgen receptor status

Characteristics	AR negative	AR positive	χ^2	<i>P</i> -value
Age				
≤ 40 years	6	23	1.336	0.567
40-70 years	194	460		
≥ 70 years	50	126		
Histotype				
Ductal and Lobular	163	419	0.894	0.335
Special types	87	190		
Grade				
Low	82	201	2.731	0.255
Intermediate	102	277		
High	66	131		
pT				
1	147	394	8.42	0.038
2	79	186		
3	9	16		
4	14	13		
pN				
0, ITC, 1MIC	115	347	8.656	0.003
1,2,3	101	186		
Vascular invasion				
No	109	275	0.116	0.733
Yes	141	334		
PgR status				
≤1%	65	114	5.263	0.022
≥1%	185	495		
Ki67				
≤10%	91	257	1.212	0.271
>10%	117	272		
HER 2				
Negative	221	525	1.791	0.408
Positive	26	74		

AR Androgen Receptor; *ITC* isolated tumor cells; *MIC* micrometastasis; *PgR* Progesterone Receptor, *HER 2* human epidermal growth factor receptor 2.

Table 3. Univariate analysis of clinical and pathological data correlated with time to relapse.

	General			Endocrine therapy			Endocrine and chemotherapy				
	% Censored	CPS at 10 years (range 0-1)	P-value	% Censored	CPS at 10 years (range 0-1)	P-value	% Censored	CPS at 10 years (range 0-1)	P-value		
Age (years)		(range 0-1)			(range 0-1)			(range 0-1)			
Age (years) ≤ 40	80.6	0.82	0.002	80	n.d.	0.008	80.8	0.83	0.003		
Age (years) 40-70	93.7	0.89		95.8	0.93		91.4	0.85			
Age (years) ≥ 70	90	0.77		92.1	0.79		76.9	n.d.			
pN											
pN 0, itc, 1mic	96.1	0.92	<0.001	95.8	0.92	<0.001	96.9	0.90	0.005		
pN 1, 2, 3	84.9	0.78		81.7	0.72		85.7	0.80			
pN 0, ITC, IMIC	91.9	0.86		92.0	0.87		93.0	0.81			
Type of surgery			<0.001			0.006			0.001		
Mastectomy	96	0.91	<0.001	97.4	0.93	<0.001	93.8	0.89	0.002		
Conservative	83.1	0.77		84.4	0.80		82	0.74			
Mastectomy	90.0	0.85		94.4	0.86		94.0	0.75			
Histotype			<0.001			<0.001			<0.001		
Conservative	74.5	0.68	0.037	82.8	0.78	0.011	66.0	0.57	0.954		
Ductal and Lobular	91.1	0.85		92.3	0.87		89.7	0.82			
Ductal and Special types Lobular	95.7	0.93		98.1	0.97		90	0.85			
Grade			0.023			0.039			0.790		
Special types	90.7	0.87	<0.001	95.2	0.92	0.002	90	0.78	0.005		
Grade 1	96.8	0.93		97.4	0.98		92.6	0.78			
Grade 2	93.8	0.89		93.7	0.88		93.9	0.91			
Grade 3	83.9	0.76	<0.001	85.1	0.79	<0.001	83.3	0.75	0.007		
pT											
pT 1	96.3	0.92		97.2	0.94		94.3	0.91			
pT 2	87.9	0.77	<0.001	87.6	0.77	<0.001	88.1	0.75	0.007		
pT 3	73.1	0.64		66.7	n.d.		76.5	0.72			
pT 4	75.9	0.78		80	n.d.		73.7	0.78			
pT 3	75.1	0.71		77.0	n.d.		70.0	0.70			
Vascular invasion											
No	97.4	0.94	<0.001	97.6	0.95	<0.001	96.7	0.92	0.018		
Yes	88.4	0.82		89.5	0.83		87.7	0.80			
Radiotherapy			<0.001			<0.001			0.066		
No	87.2	0.90	0.001	88.1	0.93	<0.001	86.3	0.76	0.184		
Yes	94.5	0.80		96.6	0.84		91.3	0.87			
PR			0.001			0.004			0.097		
Yes	88.8	0.81	0.019	93.7	0.80	0.250	81.2	0.71	0.037		
PgR 0	93.4	0.81		93	0.91		85.4	0.71			
PgR ≥1%	89.5	0.88	0.469	95.1	0.92	0.826	91.2	0.86	0.611		
HER2 ≥1%	85.0	0.78		91.9	0.91		78	0.60			
HER2 Neg	86.7	0.78	0.176	93	0.87	0.654	70.1	0.68	0.064		
HER2 Pos	90.1	0.89		94.4	0.89		90.8	0.83			
AR Neg	87.4	0.79	0.011	91.8	0.87	0.258	81	0.70	0.013		
AR Pos	70.2	0.72		93	0.85		68.8	0.62			
AR 0	86.4	0.78	<0.001	92.7	0.83	0.247	78.8	0.72	<0.001		
AR ≥1%	95.2	0.92		95.5	0.93		94.8	0.89			
KI67 0	80	0.69	0.001	88.5	0.76	0.046	69.9	0.60	0.015		
KI67 ≥1%	80.2	0.82		82.8	0.82		82.0	0.71			
KI67 0	97.3	0.95	0.002	98.4	0.96	0.002	95.1	0.92	0.178		
KI67 ≥1%	86.7	0.85		87.3	0.80		86.1	0.80			
KI67 ≥1%	95.0	0.80	<0.001	97.2	0.93	<0.001	87	0.70	0.007		
KI67 ≥1%	74.1	0.60	<0.001	80.3	0.74	<0.001	68.2	0.45	0.007		

CPS Cumulative Proportion Surviving; n.d. not done; ITC isolated tumor cells; MIC micrometastasis, PgR Progesterone Receptor; HER2 human epidermal growth factor receptor 2; AR Androgen Receptor.

Table 4 Univariate analysis of clinical and pathological data correlated with disease specific survival

CPS Cumulative Proportion Surviving; *n.d.* not done; *ITC* isolated tumor cells; *MIC* micrometastasis, *PgR* Progesterone Receptor; *HER2* human epidermal growth factor receptor 2; *AR* Androgen Receptor.

Table 5 Association of patient and tumor characteristics with time to relapse and disease specific survival among all patients with complete data for all covariates.

	Time to relapse				Disease specific survival			
	HR	95% CI	χ^2	<i>P</i> -value	HR	95% CI	χ^2	<i>P</i> -value
Age	0.390	0.232- 0.656	12.580	0.000	0.356	0.163-0.777	6.729	0.009
pN	1.599	0.919- 2.780	2.762	0.097	1.747	0.749- 4.075	1.670	0.196
Type of surgery	1.927	1.039- 3.574	4.329	0.037	2.328	0.954- 5.681	3.446	0.063
Histotype	1.435	0.888- 2.319	2.177	0.140	1.360	0.652- 2.836	0.673	0.412
Grade	1.385	0.966- 1.986	3.136	0.077	1.329	0.806- 2.192	1.246	0.264
pT	1.727	1.328- 2.246	16.654	0.000	2.089	1.455- 2.999	15.935	0.000
Vascular Invasion	2.393	1.326- 4.318	8.388	0.004	4.699	1.812- 12.182	10.133	0.001
Radiotherapy	0.924	0.515- 1.656	0.071	0.790	0.927	0.416- 2.064	0.035	0.852
Chemotherapy	0.928	0.531- 1.623	0.068	0.794	0.399	0.187- 0.854	5.595	0.018
PgR	0.600	0.356- 1.013	3.658	0.056	0.308	0.156- 0.609	11.474	0.001
HER2	1.887	1.109- 3.212	5.474	0.019	1.961	0.893- 4.303	2.817	0.093
AR	0.458	0.296- 0.710	12.175	0.000	0.259	0.139- 0.482	18.184	0.000
KI67	2.234	1.297- 3.848	8.394	0.004	1.686	0.790- 3.597	1.826	0.177

HR Hazard Ratio; *CI* Confidence Interval; *PgR* Progesterone Receptor, *HER2* human epidermal growth factor receptor 2; *AR* Androgen Receptor.

Table 6 Association of patient and tumor characteristics with time to relapse and disease specific survival among patients treated by endocrine therapy with complete data for all covariates

PATIENTS TREATED BY ENDOCRINE THERAPY

	Time to relapse				Disease specific survival			
	HR	95% CI	χ^2	<i>P</i> -value	HR	95% CI	χ^2	<i>P</i> -value
Age	0.241	0.091- 0.642	8.102	0.004	0.399	0.111- 1.426	2.000	0.157
pN	1.925	0.658- 5.626	1.431	0.232	1.877	0.544- 6.472	0.993	0.319
Type of surgery	2.161	0.613- 7.624	1.435	0.231	3.263	0.751- 14.188	2.488	0.115
Histotype	1.718	0.677- 4.364	1.297	0.255	0.968	0.251- 3.726	0.002	0.962
Grade	0.918	0.518- 1.629	0.085	0.771	1.038	0.498- 2.166	0.010	0.921
pT	1.838	1.079- 3.131	5.023	0.025	2.410	1.285- 4.522	7.508	0.006
Vascular Invasion	3.738	1.367- 10.221	6.599	0.010	3.564	0.946- 13.423	3.527	0.060
Radiotherapy	0.596	0.176- 2.022	0.689	0.407	0.934	0.238- 3.664	0.009	0.922
PgR	1.042	0.355- 3.063	0.006	0.940	0.506	0.150- 1.703	1.210	0.271
HER2	0.912	0.234- 3.553	0.018	0.894	0.741	0.143- 3.855	0.127	0.722
AR	0.427	0.190- 0.959	4.246	0.039	0.413	0.150- 1.137	2.928	0.087
KI67	2.684	0.969- 7.438	3.604	0.058	1.566	0.477- 5.142	0.546	0.460

HR Hazard Ratio; CI Confidence Interval; PgR Progesterone Receptor, HER2 human epidermal growth factor receptor 2; AR Androgen Receptor.

Table 7 Association of patient and tumor characteristics with time to relapse and disease specific survival among patients treated by endocrine and chemotherapy with complete data for all covariates

PATIENTS TREATED BY ENDOCRINE AND CHEMOTHERAPY

	Time to relapse				Disease specific survival			
	HR	95% CI	χ^2	<i>P</i>	HR	95% CI	χ^2	<i>P</i>
Age	0.489	0.253- 0.947	4.505	0.034	0.280	0.091- 0.866	4.881	0.027
pN	1.721	0.849- 3.486	2.269	0.132	1.367	0.362- 5.170	0.213	0.645
Type of surgery	1.990	0.966- 4.096	3.486	0.062	1.666	0.590- 4.706	0.927	0.336
Histotype	1.376	0.752- 2.516	1.074	0.300	2.353	0.853- 6.494	2.730	0.098
Grade	2.030	1.218- 3.385	7.372	0.007	2.281	0.978- 5.318	3.645	0.056
pT	1.625	1.190- 2.220	9.327	0.002	2.220	1.354- 3.641	9.992	0.002
Vascular Invasion	1.969	0.914- 4.242	2.994	0.084	11.298	1.847- 69.091	6.887	0.009
Radiotherapy	1.062	0.529- 2.131	0.029	0.865	0.652	0.234- 1.822	0.664	0.415
PgR	0.466	0.248- 0.878	5.589	0.018	0.176	0.068- 0.455	12.883	0.000
HER2	2.211	1.216- 4.022	6.758	0.009	3.022	1.138- 8.021	4.928	0.026
AR	0.444	0.258- 0.765	8.555	0.003	0.135	0.054- 0.337	18.339	0.000
KI67	1.842	0.958- 3.543	3.350	0.067	1.575	0.548- 4.526	0.710	0.399

HR Hazard Ratio; CI Confidence Interval; PgR Progesterone Receptor, HER2 human epidermal growth factor receptor 2; AR Androgen Receptor.

Table 8 Association of patient and tumor characteristics with time to relapse and disease specific survival among 343 patients with luminal B cancer with complete data for all covariates

	PATIENTS WITH LUMINAL B BREAST CANCER							
	Time to relapse				Disease specific survival			
	HR	95% CI	χ^2	<i>P-value</i>	HR	95% CI	χ^2	<i>P-value</i>
Age	0.290	0.162- 0.520	17.242	0.000	0.354	0.148- 0.846	0.846	0.019
pN	1.638	0.846- 3.169	2.143	0.143	2.391	0.871- 6.562	2.864	0.091
Type of surgery	1.763	0.810- 3.835	2.044	0.153	2.525	0.923-6.905	3.256	0.071
Histotype	1.367	0.771- 2.425	1.145	0.285	1.081	0.434- 2.694	0.028	0.867
Grade	1.197	0.791- 1.810	0.724	0.395	1.130	0.625-2.043	0.163	0.686
pT	1.826	1.367- 2.439	16.595	0.000	2.008	1.339- 3.011	11.377	0.001
Vascular Invasion	2.203	1.093- 4.438	4.884	0.027	6.836	1.737- 26.908	7.560	0.006
Radiotherapy	0.753	0.353- 1.607	0.539	0.463	1.009	0.395- 2.581	0.000	0.985
Chemotherapy	0.780	0.398- 1.530	0.521	0.470	0.351	0.142- 0.870	5.114	0.024
PgR	0.712	0.381- 1.328	1.142	0.285	0.351	0.206- 1.066	3.270	0.071
AR	0.521	0.306- 0.888	5.743	0.017	0.276	0.130- 0.588	11.135	0.001

HR Hazard Ratio; *CI* Confidence Interval; *PgR* Progesterone Receptor; *AR* Androgen Receptor.